A Novel QTL for Root-Knot Nematode Resistance is Identified from a South African Sweet Sorghum Line

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ABSTRACT

Southern root-knot nematodes, *Meloidogyne incognita*, feed on the underground portions of hundreds of plant species and affect nutrient partitioning and water uptake of the host plants. Sorghum (*Sorghum bicolor*) is often not significantly damaged by southern root-knot nematodes (RKN) but some sorghum genotypes support greater population densities of RKN than other genotypes. These higher nematode populations increase the risk of damage to subsequently planted susceptible crops. A previous study identified a major quantitative trait locus (QTL) for RKN resistance on sorghum chromosome (chr.) 3. To maintain long-term resistance, multiple resistance genes should be pyramided in a cultivar. In this study, we identified a new source of RKN

resistance, created a mapping population, and identified single-nucleotide polymorphism markers using genotyping-by-sequencing of the segregating population. Use of single-marker analysis and composite interval mapping identified a single QTL on chr. 5 that was associated with egg number and egg number per gram of root from the resistant sweet sorghum line PI 144134. This region on chr. 5 and the prior QTL on chr. 3 can be potentially moved from PI 144134 and Honey Drip, respectively, into elite sorghum germplasm via marker-assisted selection for more durable resistance.

Keywords: genetics and resistance, nematology.

Southern root-knot nematodes (RKN) feed on a wide host range of plants, have worldwide distribution in mild temperate areas to tropical and subtropical regions, and reproduce by parthenogenesis (Donkers-Venne et al. 2000; Favery et al. 2016). In terms of crop damage, RKN are considered the most economically important plant-parasitic nematodes (Danchin et al. 2013), feeding on the roots, bulbs, and tubers of thousands of plant species. Economically important crops, including cotton (*Gossypium hirsutum* L.), tobacco (*Nicotiana tabacum* L.), food legumes, vegetables, spices, and coffee (*Coffea* spp.), can be damaged severely by RKN (Choi et al. 2017; Ramana and Eapen 1995). Damage to the underground portions of the plants reduces water and nutrient uptake, thus reducing plant vigor and yield.

To control RKN, farmers frequently use soil fumigants or other nematicides, crop rotation, and resistant cultivars. Some of the commonly used nematicides such as 1,3 dichloropropene (Telone II, Curfew) and fenamiphos (Nemacur), which were used as alternatives to methyl bromide, have been banned by the European Union and the U.S. Environmental Protection Agency, respectively, due to high toxicity to animals and the environment (Crow 2005; Danchin et al. 2013). The most sustainable control methods with the least potential for human harm and environmental damage are crop rotation and the use of RKN-resistant cultivars. Unfortunately, the

use of resistant varieties is limited by the number of available resistant genotypes, and resistance, especially monogenic resistance, can be overcome by the nematodes with time (Favery et al. 2016).

Resistance to *Meloidogyne* spp. has been found in many plant species and is controlled by mostly a small number of genes. In cowpea (*Vigna unguiculata* L. Walp.), resistance to *Meloidogyne incognita* and *M. javanica* isolates in lines IT84S-2049, IT93K-503-1, and CB27 was conferred by one quantitative trait locus (QTL) with a major effect (Huynh et al. 2016). Similarly, resistance to *M. incognita* in pearl millet (*Pennisetum glaucum* (L.) R. Br. line Tift454 is conferred by one major QTL (Liu 2012). Resistance to *M. incognita* in soybean (*Glycine max* L. Merr.) line PI 567516C was found to be conferred by three QTLs (Jiao et al. 2015) and resistance to *M. graminicola* in rice (*Oryza sativa* L.) variety Bala is conferred by four QTLs (Shrestha et al. 2007).

Sorghum, including sweet sorghum, is often not significantly damaged by RKN but some sorghum genotypes have been shown to support greater population densities of RKN (Davis et al. 2015; McSorley and Gallaher 1993). These higher populations act as future inoculum, increasing the risk of damage to subsequent susceptible crops. A recent study examining 29 sorghum hybrids for RKN resistance found that 28 of the sorghum hybrids were susceptible and, thus, the potential for high densities of RKN accumulating in the soil exists (Xavier-Mis et al. 2017). Because farmers often utilize crop rotation and may plant cotton, corn (*Zea mays* L.), soybean, or other RKN-susceptible crops after sorghum (Xavier-Mis et al. 2017), it is important to understand the location and the number of genes involved in RKN resistance in sorghum. This information is valuable in creating new sorghum hybrids with RKN resistance using marker-assisted selection.

RKN resistance in sorghum has been examined previously using the resistant sweet sorghum cultivar Honey Drip (PI 641821) (Harris-Shultz et al. 2015a). A major QTL on chromosome (chr.) 3 was associated with a reduction in total eggs (57% phenotypic

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variance) and eggs per gram of root (70% of the phenotypic variance). The identification of multiple sources of resistance in sorghum, followed by the pyramiding of these genes into elite germplasm using marker-assisted selection, should confer more durable resistance to RKN. In this study, we sought to identify QTLs associated with RKN resistance that contain resistance genes different than Honey Drip.

MATERIALS AND METHODS

Identification of lines potentially containing a new RKN resistance gene. To identify sorghum lines that potentially have a resistance gene different than Honey Drip, Colman (PI 641810), Leoti-Peltier (PI 642999), Honey Drip (PI 641821), MN 395 (PI 144134), Dale (PI 651495) (Broadhead and Coleman 1973), Top 76-6 (also known as Topper; PI 583832) (Day et al. 1995), M 81E (PI 653411) (Broadhead and Zummo 1983), Entry 22 (Harris-Shultz et al. 2015b), Collier (PI 641862), and GT-IR7 (PI 602445) (Widstrom 1998) were genotyped in the sorghum chr. 3 region that was previously found to be associated with reduced egg number and egg number per gram of root (Harris-Shultz et al. 2015a). The sorghum simple sequence repeat (SSR) markers RKNP135, RKNP194, RKNP259, RKNP342, RKNP402, RKNP465, and RKNP529 (Punnuri et al. 2017) (Supplementary Table S1) were amplified in these lines using the previously described reaction and thermocycler conditions (Harris-Shultz et al. 2015a). Polymerase chain reaction (PCR) product (2 μ l) was combined with 5 μ l of Blue Stop (LI-COR Biosciences, Lincoln, NE, U.S.A.) and 0.35 μ l of this mixture was loaded on a 6.5% acrylamide gel using a LI-COR Biosciences 4300 DNA Analyzer. Gel images were scored visually. The marker data were imported into NTSYSpc (Rohlf 2008). Genetic similarity between each pair of accessions was calculated using the SIMQUAL module using the DICE coefficient of similarity (Nei and Li 1979). A dendrogram was generated from the similarity matrix by using the unweighted pair-group method with arithmetic mean procedure in the SAHN module of NTSYSpc. Bootstrap resampling was performed using the software program

FreeTree (Hampl et al. 2001) with 1,000 bootstrap repetitions; bootstrap values >50% were placed on the resulting dendrogram.

Development of an F₂ population segregating for RKN resistance. An F₂ population (PI 144134 $\times$ Collier) was created by hand emasculating florets of PI 144134 and transferring pollen from Collier the following day, for 3 days, to produce potential F₁ plants. Because sorghum has a high rate of self-pollination and errors can occur during anther removal, plants were confirmed as being a product of the cross by genotyping using the polymorphic SSR markers RKNP194, RKNP259, RKNP342, and RKNP529 (Harris-Shultz et al. 2015a; Punnuri et al. 2017), as previously described (Harris-Shultz et al. 2015a). Plants that were identified as true crosses were then self-pollinated, and the resulting F₂ seed from a single head was planted for subsequent phenotyping and genotyping.

Phenotyping F₂ plants for RKN egg number, root weight, and Brix. The PI 144134 $\times$ Collier F₂ population was phenotyped for RKN response by growing 15 plants of PI 144134, 34 plants of Collier, 198 F₂ plants, and 2 plants of the sorghum cultivar ES 5200 (Blade Energy Crops, Ceres, Inc., Thousand Oaks, CA, U.S.A.), a known susceptible hybrid used for forage, in a temperature-controlled greenhouse in steam-pasteurized soil (Tifton loamy sand) in 15-cm-diameter pots with 1 plant/pot. Due to a low number of F₁ plants being obtained, F₁ seed was remade and the F₁ plants were phenotyped in a second experiment consisting of 14 plants of Collier, 13 plants of PI 144134, and 11 plants of the PI 144134 $\times$ Collier F₁. Plants that had root weights of <10 g were excluded from analysis to eliminate plants that had too little root tissue to allow normal amounts of RKN reproduction.

Twenty-eight days after planting, each pot was infested with 8,000 eggs of *M. incognita* race 3 when most plants were still in the vegetative growth stage 1. Inoculum was collected from eggplant (*Solanum melongena* L.) roots by agitating roots in 0.5% sodium hypochlorite solution for 2 min (Hussey and Barker 1973) 1 h or less before inoculation. Inoculum was distributed into two holes (approximately 2.5 cm deep) and covered with soil, and pots were then watered immediately.

Fig. 1. Unweighted pair-group method with arithmetic mean dendrogram of data from seven simple sequence repeat markers within the sorghum root-knot nematode resistance quantitative trait locus region identified from the cultivar Honey Drip. Ten sorghum lines that were resistant, moderately resistant, or susceptible to root-knot nematodes were genotyped using these markers.

At harvest, 8 weeks after inoculation, when most plants were at anthesis, the stem of each plant was cut at the soil line and leaf tissue was removed for DNA extraction and placed in the -80°C freezer. To determine Brix content, the stems were folded in half and crushed using a home-built billet crusher. The crusher consists of a piece of steel channel mounted on a stand at an angle of approximately 45° . A narrower piece of square steel tubing is connected to the lower end of the channel by a hinge. The billet is placed between the tubing and channel, and pressure is applied manually to extract the juice, which runs down the channel for collection. The degrees Brix was measured from the extracted juice using a digital refractometer (Refracto 30GS; Mettler-Toledo, Schwerzenbach, Switzerland). One degree Brix is equivalent to 1 g of sucrose in 100 g of solution.

After the stems were removed, nematode eggs were extracted from all roots in a pot. Roots were gently washed free of soil, cut into approximately 5-cm pieces, and agitated in a 1% sodium hypochlorite solution in a 1-liter flask for 4 min. Eggs were collected, rinsed with tap water on nested 150- over 25- μm -pore sieves, and counted under a microscope.

DNA extraction and single-nucleotide polymorphism genotyping. DNA was extracted from each parent and from the 198 F_2 plants using the GenJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, U.S.A.). The parents of the population and 187 F_2 progeny were selected for genotyping-by-sequencing (GBS) based on having data for root weight, egg counts, and Brix. Tunable GBS (Ott et al. 2017), which has a higher genome reduction level than conventional GBS, was performed by Data2Bio (Ames, IA, U.S.A.) using five runs on an Ion Proton Instrument (Thermo Fisher Scientific) generating an average of 3 million reads per sample. Bases with Phred quality scores of <15 of 40 were trimmed. The *Sorghum bicolor* genome was downloaded from Joint Genome Institute Phytozome v12 website (Goodstein et al. 2012) and was used as the reference genome. Trimmed reads from each sample were aligned to the sorghum reference genome using the Genomic Short-read Nucleotide Alignment Program (Wu and Nacu 2010).

Any nucleotide site that was polymorphic in at least one sample as compared with the reference genome sequence was considered a single-nucleotide polymorphism (SNP). SNPs were retained if the most common allele for a homozygous SNP or the sum of the reads of the two most common alleles for a heterozygous SNP accounted for at least 80% of all the aligned reads covering that nucleotide position, if at least five unique reads supported the most common allele (or the two most common alleles for heterozygotes), and if each polymorphic base had a Phred base quality value ≥ 20 . Polymorphisms in the first and last 3 bp of each read were ignored. SNPs were further filtered using a minimum call rate $\geq 50\%$, an allele number of 2, a minor allele frequency of ≥ 0.35 , and a heterozygosity range of 35 to 65%. SNP genotype calls were provided by Data2Bio.

Correlations and QTL analysis. For egg number, egg number per gram of root, and Brix, data were normalized by using a $\log_{10}(x + 1)$ transformation. The Pearson's correlation coefficient between each of the four traits measured was calculated in Excel 2010 (Microsoft Corp, Redmond, WA, U.S.A.). The strength of the correlation coefficients was determined by using the Dancey and Reidy (2004) categorization.

For single-marker analysis (SMA), the raw Data2Bio genotypes were filtered to remove sites with $>55\%$ heterozygosity across individuals, because preliminary inspection of the data indicated that these were likely false SNPs due to misalignment of paralogs.

TABLE 1. Pearson's correlation coefficients between root weight (Root Wt), egg number (LogEggs), eggs per gram of root (LogEggRoot), and Brix (LogBRIX) measured on 187 PI 144134 $\times$ Collier F_2 plants^a

Trait	Root Wt	LogEggs	LogEggRoot	LogBRIX
Root Wt	1	...	...	...
LogEggs	0.208**	1	...	...
LogEggRoot	0.212**	0.978**	1	...
LogBRIX	0.548**	0.066	0.104	1

^a Asterisks (**) indicate $P < 0.01$.

Fig. 2. Frequency distributions for **A**, root-knot nematode egg number; **B**, eggs per gram of root; **C**, root weight; and **D**, Brix in a PI 144134 $\times$ Collier F_2 mapping population.

Genotypes were then imputed with FSFhap (Swarts et al. 2014), except for chr. 5 and 8, which the algorithm was unable to complete (likely due to misplaced markers; data not shown). Redundant markers were then removed with R (R Core Team 2018). To test whether transformation of the phenotype data would improve mapping power, we estimated the heritability of raw and log-transformed values for all four phenotypes, along with egg number and egg number per gram of root when divided into either two (resistant and susceptible) or three (resistant, intermediate, and susceptible) groups. Note that the log transformation was not $\log(x + 1)$; thus, individuals with a count of 0 were set to missing; this was done intentionally because of the extreme skew the 0-count individuals introduced to the distribution, and because they would still be captured by the two- and three-category divisions of the phenotypes. Narrow-sense heritability was estimated using a mixed linear model in TASSEL (Bradbury et al. 2007) to get genetic variance components; this identified the raw root weight, raw Brix, log-transformed egg number, and log-transformed egg number per gram of root as the most robust version of each trait (data not

shown). Finally, SMA was performed in TASSEL using a general linear model with 10,000 random permutations of the data to determine empirical P values. The entire SMA workflow is available at https://github.com/wallacelab/2018_sorghum_nematodes.

RESULTS

Identification of PI 144134 as potentially containing a new RKN resistance gene. In a previous study (Harris-Shultz et al. 2015a), a major QTL on chr. 3 was associated with a reduction in total eggs and eggs per gram of root in the sweet sorghum cultivar Honey Drip. To confer more durable RKN resistance via pyramiding of resistance genes in sorghum lines, additional sorghum lines with RKN resistance were examined with SSR markers located in the Honey Drip QTL region to potentially find alternative resistance genes. Sweet sorghum lines Colman, Leoti-Peltier, Honey Drip, and PI 144134 had been previously found to be resistant to RKN (Davis and Anderson 2012). Dale, Top 76-6 (Topper), and M 81E had been found to be moderately resistant,

Fig. 3. Single-marker analysis using single-nucleotide polymorphisms (SNPs) for the indicated traits in the PI 144134 × Collier F₂ mapping population. Counts for number of eggs and eggs per gram of root were log-transformed prior to mapping because of the highly skewed distribution of these phenotypes (see Materials and Methods for details). Chromosome (chr.) position is shown on the x-axis, while the y-axis shows the $-\log_{10} P$ value for each point. Points are colored according to their empirical P value based on 10,000 random permutations of the data. A small number of isolated significant hits for egg counts on chr. 1, 3, and 6 appear to be misplaced markers, because including the peak SNP on chr. 5 as a covariate makes these points nonsignificant (data not shown).

whereas Entry 22, Collier, and GT-IR7 had been found to be susceptible to RKN (Davis and Anderson 2012). Seven SSR markers located in the QTL region associated with reduced egg number and egg number per gram of root were used to genotype the 10 lines. Twenty-eight fragments were generated and resistant lines Colman, Leoti-Peltier, and Honey Drip had similar haplotypes (83 to 100% genetic similarity) for this region (Fig. 1). Resistant line PI 144134 grouped with lines that were moderately resistant and susceptible and, thus, PI 144134 may have an alternative source of resistance. This line was selected for crossing with susceptible line Collier to create an F₂ mapping population.

Phenotypic summary of the PI 144134 × Collier F₂ population. *Total eggs.* Total eggs for the resistant parent PI 144134 ranged from 0 to 2,400 eggs whereas the susceptible parent Collier ranged from 18,000 to 295,800 eggs. The F₂ plants ranged from 0 to 243,600 eggs and the frequency distribution for total eggs is shown (Fig. 2A). The total egg data for the F₂ plants displays a nonnormal distribution (skewness = 2.73, kurtosis = 7.26), with individuals skewed toward the resistant parent. Using a cutoff of ≤12,000 eggs for resistant and ≥21,000 eggs for susceptibility, 150 F₂ plants were resistant and 40 F₂ plants were susceptible. A χ^2 test was used to test the goodness of fit to a 3:1 ratio, assuming that a single dominant gene restricts total eggs and supports a single dominant gene ($\chi^2 = 1.579$, df = 1, $P = 0.2089$). For the F₁ test, total eggs for the resistant parent PI 144134 ranged from 0 to 10,800

eggs, the susceptible parent Collier ranged from 22,200 to 247,800 eggs, and the F₁ plants ranged from 4,800 to 36,600 eggs.

Eggs per gram of root. Eggs per gram of root ranged from 0 to 229 eggs/g of root for PI 144134 and 1,362 to 19,720 eggs/g of root for Collier. The F₂ plants ranged from 0 to 18,119 eggs/g of root. The F₂ frequency distribution was nonnormal (skewness = 2.98, kurtosis = 10.12) and was skewed toward the resistant parent (Fig. 2B). Similar to egg number, a break in the F₂ data was seen and <871 eggs/g of root was labeled as resistant, whereas >1,328 eggs/g of root was labeled as susceptible. Thus, 150 F₂ plants were resistant and 40 F₂ plants were susceptible. A χ^2 test was used to test the goodness of fit to a 3:1 ratio, assuming that a single dominant gene restricts total eggs per gram of root and supports a single dominant gene ($\chi^2 = 1.579$, df = 1, $P = 0.2089$). For the F₁ test, eggs per gram of root ranged from 0 to 1,714 eggs/g of root for PI 144134, 1,708 to 11,939 eggs/g of root for Collier, and 293 to 3,327 eggs/g of root for the F₁ plants.

Root weight. Root weight for PI 144134 and Collier ranged from 8.4 to 23.3 and 4.4 to 21.2 g, respectively. Using a *t* test, these parental lines were not significantly different ($t = 1.12$, df = 16, $P = 0.14$) for root weight. The F₂ plants ranged from 7.2 to 32.7 g of root weight and the F₂ frequency distribution was normally distributed (skewness = 0.835, kurtosis = 0.668) (Fig. 2C). For the F₁ test, root weight for PI 144134 and Collier ranged from 10.0 to 16.4 and 12.3 to 42.6 g, respectively. Root weight for the F₁ plants ranged from 11.0 to 20.3 g.

Fig. 4. Composite interval mapping was performed for all traits in R/qtl (Broman et al. 2003). The dotted line represents the 95% significance threshold determined by 1,000 random permutations of the data. Brix and root weight show no significant hits, while number of eggs and eggs per gram of root both show a single peak on Chromosome 5.

TABLE 2. Identification of quantitative trait loci using composite interval mapping in the PI 144134 × Collier F₂ population^a

Trait	LG/cM	SNP	Log of odds	Additive ^b	Dominance ^b	R ²	Chr./bp
EggRoot (log)	5/45.97	s1221	42.92	-3.68 (39.6×)	-1.44 (4.2×)	0.72	5/5,608,057
EggNo. (log)	5/45.97	s1221	44.37	-3.77 (43.4×)	-1.36 (3.9×)	0.73	5/5,608,057

^a Abbreviations: LG = linkage group, cM = centimorgans, SNP = single-nucleotide polymorphism, and Chr. = chromosome.

^b The sign of the estimated additive and dominance effects are associated with the allele from the female (resistant) parent. Values are on a natural log scale, with the associated fold-change shown in parentheses.

Brix. The Brix values between PI 144134 and Collier were significantly different using a *t* test ($t = 1.97$, $df = 16$, $P = 0.03$). The Brix values for PI 144134 ranged from 1.5 to 8.5 °Bx, Collier ranged from 2.7 to 11.6 °Bx, and the F₂ progeny ranged from 1.8 to 12.6 °Bx. The F₂ frequency distribution is multimodal with peaks seen at 2.63, 4.29, and 7.62 °Bx and has a light tailed distribution (skewness = 0.584, kurtosis = -0.387) (Fig. 2D). Brix values were not taken on the F₁ plants.

Correlation among phenotypic traits. A moderate correlation was seen between root weight and Brix (Table 1) ($r = 0.55$). Similarly, a strong correlation was seen between egg number and eggs per gram of root (Table 1) ($r = 0.98$).

Marker by trait association analysis. Single-marker association was used to identify marker-trait associations between 911 nonredundant SNP markers and four traits from 187 F₂ progeny. Of all the traits examined, only egg number and egg number per gram of root had a significant marker-trait association, with peak association at a single marker, s1221, on chr. 5 at 5,608,057 bp (Fig. 3). Presence of the PI 144134 allele in the homozygous or heterozygous condition for this SNP resulted in F₂ progeny having between 0 to 12,000 eggs and 0 to 586 eggs/g of root. Presence of the Collier allele in the homozygous condition for SNP s1221 resulted in the F₂ progeny having egg numbers that ranged between 12,000 to 243,600 eggs and 870 to 17,177 eggs/g of root. Three progeny (F2_71, F2_165, and F2_216) are outliers whose egg count is in the range of the opposite genotype; these may be genotyping errors and, thus, are excluded from the above ranges.

Composite interval mapping (CIM) was performed in R/qtl (Broman et al. 2003) after calculating genetic map distances and removing misplaced markers that created large gaps in the map due to errant segregation. CIM also confirmed that a single QTL with its peak log of odds at s1221 (Fig. 4) is associated with the number of RKN eggs and number of eggs per gram of root. No QTLs were detected for Brix or root weight (Fig. 4). The QTL effect for both the number of eggs and eggs per gram of root was high ($R^2 = 72$ to 73%) and both overlapping QTLs showed partial dominance ($D/A = 0.36$ to 0.39) (Stubber et al. 1987) (Table 2).

DISCUSSION

The moderate correlation between root weight and Brix (Table 1) for the PI 144134 × Collier F₂ population had been previously seen in the Honey Drip × Collier RKN F₂ mapping population (Harris-Shultz et al. 2015a). Although correlations do not imply causation, one could speculate that, for sweet sorghum plants, healthier plants with larger root weights may have a better ability to accumulate sugars.

A frequency distribution for root weight and Brix (Fig. 2) indicated that these traits are controlled by a large number of genes. In contrast, the number of eggs or the number of eggs per gram of root is controlled by a small number of genes (Fig. 2). Genotyping in the Honey Drip QTL region, *QTL-Sb.RKN.3.1*, of 10 inbred lines with various responses to RKN revealed that PI 144134 may have a novel resistance gene. Use of SMA and CIM identified a single QTL on chr. 5 that is associated with the number of RKN eggs or the number of eggs per gram of root (Figs. 3 and 4). Thus, we were successful in identifying a new resistance locus associated with egg number and egg number per gram of root from PI 144134 which is different than the major gene found from Honey Drip on sorghum chr. 3. This new QTL will be here named *QTL-Sb.RKN.5.1*.

Plant resistance proteins recognize the corresponding pathogen avirulence proteins through direct interaction or indirectly through detection of changes in the host protein targets (Dodds et al. 2006). This recognition event between resistance and avirulence proteins causes host defense responses, mainly manifested as a hypersensitive response, which is the rapid death of the invaded cell and, in some cases, some of the surrounding cells (Dodds et al. 2006). Resistance gene analogs can be classified as nucleotide-binding

leucine-rich repeat (NBS-LRR), transmembrane LRR proteins, and other modes of resistance such as pentatricopeptide repeats and peroxidases (Sekhwal et al. 2015). Many of the genes that have been found to be involved with nematode resistance in plants include those that encode members of the NBS-LRR family of proteins (Ernst et al. 2002; Lagudah et al. 1997; Milligan et al. 1998). Similarly, the Hs1^{Pro-1} gene from sugar beet (*Beta vulgaris* L.) which confers resistance to the beet cyst nematode (*Heterodera schachtii*) has imperfect LRRs and a putative transmembrane domain (Cai et al. 1997). In soybean, the loci *Rhgl* and *Rhg4*, which confer resistance to *H. glycines* type 0, encode proteins with extracellular LRRs, a transmembrane domain, and a cytosolic serine-threonine kinase domain. Examination of the chr. 5 QTL area from 5,170,367 to 5,790,187 bp (Supplementary Table S2) revealed one gene that contains an N-terminal LRR and a serine-threonine protein kinase domain, Sobic.005G055900, near the peak QTL region starting at 5,657,820 bp. There are also 11 additional genes encoding an LRR domain nearby but further away from the peak SNP. Further examination using root gene expression differences between the parental lines or further fine-mapping may help narrow the number of candidate genes.

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